Page 13: Remove second paragraph and insert therefor:

In a preferred embodiment, the domain capable of binding selectively a defined sequence of DNA present in the molecules of the invention is derived from the tetR or Cro proteins. It is most particularly advantageous to use the complete tetR or Cro proteins.

g2

Page 14: Remove first paragraph, and insert therefor:

4 or 5 leucines every 7 amino acids. This periodicity allows the localization of the leucines roughly at the same position on the α helix. The dimerization is sustained by hydrophobic interactions between the same chains of the leucine of two contiguous zipper domains (Vogt et al., Trends in Bioch. Science 14 (1989) 172). The SH2 domains can be used to form an oligomer with any transactivator or transactivating complex comprising the corresponding proline-rich peptide (Pawson et al., Current Biology 3 (1993) 434). It is also possible to use protein regions known to induce oligomerization, such as especially the C-terminal region of the p53 protein. The use of this region makes it possible to recruit selectivity the p53 proteins present in a cell. A p53 region between amino acids 320-290 (SEQ ID No. 3) is preferably used within the framework of the invention.

g2

Page 16: Remove the third paragraph, and insert therefor:

The DNA binding domain and the transactivator-binding domain are generally linked to each other through an arm. This arm generally consists of a peptide which confers sufficient flexibility for the two domains of the molecules of the invention to be functional autonomously. This peptide is generally composed of uncharged amino acids,

g3

which do not interfere with the activity of the molecules of the invention, such as for example glycine, serine, tryptophan, lysine or proline. The arm generally comprises from 5 to 30 amino acids and, preferably, from 5 to 20 amino acids. Examples of peptide arms which can be used for the construction of the molecules are for example:

93 cont'd

-GGGGSGGGGSGGGGS (SEQ ID No. 5)

-PKPSTPPGSS (SEQ ID No. 6).

Page 17: remove the first paragraph, and insert therefor:

94 Sequence is CCCAAGCCCAGTACCCCCCCAGGTTCTTCA (SEQ ID No. 7).

Page 17: remove third paragraph and insert therefor:

95 This type of molecule comprises:

- a domain for binding to a transactivator consisting of a single-chain antibody, a tag peptide sequence recognized by a monoclonal antibody allowing the immunological detection of the molecule. This sequence may be for example the VSV epitope of the MNRLGK sequence (SEQ ID No. 8) whose coding sequence is ATGAACCGGCTGGGCAAG (SEQ ID No. 9) or the myc epitope of the sequence EQKLISEEDLN (SEQ ID No. 10) whose coding sequence is GAACAAAAACTCATCTCAGAAGAGGATCTGAAT (SEQ ID No. 11), which is recognized by the antibody 9E10
- a peptide arm of sequence SEQ ID No. 6 (Hinge) and
- a DNA-binding domain consisting of the TET or Cro protein. Preferably, ScFv is directed against a p53 protein.

Page 20: remove first paragraph, and insert therefor:

Consists in a nucleic acid sequence encoding a chimeric molecule as defined above. It is advantageously a DNA, especially a cDNA, sequence. It may also be an RNA. The sequences of the invention are generally constructed by assembling, within a cloning vector, the sequences encoding the various domains according to conventional molecular biology techniques. The nucleic acid sequences of the invention can optionally be modified chemically, enzymatically or genetically, in order to generate domains which are stabilized, and/or multifunctional, and/or of reduced size, and/or with the aim of promoting their location in such or such intracellular compartment. Thus, the nucleic acid sequences of the invention may comprise sequences encoding nuclear localization peptides (NLS). In particular, it is possible to fuse the sequences of the invention with the sequence encoding SV40 virus NLS, whose peptide sequence is the following: PKKKRKV (SEQ ID No. 12) (Kalderon et al., Cell 39 (1984) 499).

Page 22: remove the second paragraph, and insert therefor:

As regards the minimal transcriptional promoter, it is a promoter whose activity depends on the presence of a transactivator. Because of this, in the absence of the chimeric molecule, the promoter is inactive and the gene is not or is barely expressd. On the other hand, in the presence of a chimeric molecule, the transactivator or transactivating complex recruited makes it possible to induce the activity of the minimal promoter and thus the expression of the gene of interest. The minimal promoter generally consists of an INR or a TATA box. These components are indeed the minimum components necessary for the expression of a gene in the presence of a

transactivator. The minimal promoter can be prepared from any promoter by genetic modification. By way of preferred example of a candidate promoter, there may be mentioned the promoter of the thymidine kinase gene. Advantageous results have more precisely been obtained with a minimal promoter derived from the TK promoter that comprises the TATA box of the thymidine kinase promoter.

g7 cancel

Page 23: remove first paragraph and insert therefor:

g8

from human CMV. Any conventional promoter can however be used such as, for example the promoter of the genes encoding chloramphenicol acetyltransferase, β-galactosidase or alternatively luciferase

Page 23: remove third paragraph and insert therefor:

g9

Still more preferably, the minimal promoter includes the TATA box of the thymidine kinase gene promoter.

Page 31: remove third paragraph and insert therefor:

g10

Among the synthetic vectors developed, the cationic polymers of the polylysine, $(LKLK)_n$ (SEQ ID NO:13), $(LKKL)_n$ (SEQ ID NO:14), poly(ethylenimine) and DEAE dextran type or alternatively the cationic lipids or lipofectants are the most advantageous. They posses the properties of condensing the ADN and of promoting its association with the cell membrane. Among the latter, there may be mentioned the lipopolyamines (lipofectamine, transfectam and the like) and various cationic or neutral lipids (DOTMA,

DOGS, DOPE and the like) more recently, the concept of targeted transfection, mediated by a receptor, has been developed, which takes

Page 39: remove the first paragraph and insert therefor:

1.1 Construction of the plasmid pTETop7/CAT

The plasmid pTETop7/CAT contains the following components (Figure 3):

- a regulatory sequence consisting of a sequence for interaction with the tetracycline repressor TetR composed of 7 repeated Tetop motifs (SEQ ID No. 1);
- a minimal promoter derived from the promoter of the thymidine kinase gene that includes the TATA box of the thymidine kinase gene promoter;
- the sequence encoding chloramphenicol acetyltransferase (CAT) under the control of the said minimal promoter.

Page 40: remove the first, second and third paragraphs, and insert therefor:

promoter of the thymidine kinase gene that includes the TATA box of the thymidine kinase gene promoter;

- the sequence encoding chlorampenicol acetyltransferase (CAT) under the control of the said minimal promoter.

This plasmid was constructed in the following manner: the sequence OR3 for interaction with the Cro repressor was synthesized artificially. For that, the following two oligonucleotides were synthesized:

Oligo 5533 (SEQ ID No. 15): 5'- GATCCTATACACCGCAAGGGATAA-3'

Oligo 5534 (SEQ ID No. 16): 3'-GATAGTGGCGTTCCCTATTTCGA-5'

These two oligonucleotides were then hybridized in order to reconstitute the double-stranded sequence OR3 bordered by sequences allowing its oriented cloning as follows:

GATCCTATACACCGCAAGGGATAA (SEQ ID No. 15)
GATAGTGGCGTTCCCTATTTCGA (SEQ ID No. 16)

Page 42, remove the first and second paragraphs, and insert therefor:

3.1. Construction of a plasmid comprising a sequence ScFv-myc-Hinge-TetR or Cro (Figures 5A and 6) encoding the anti-p53 ScFv was first cloned into a plasmid of the pUC19 type. The sequence encoding the VsV epitope (SEQ ID No. 8) or the myc epitope (SEQ ID No. 10) is inserted downstream of the fragment (Figure 6).

The sequence encoding the TetR and Cro proteins were then obtained as follows:

- The sequence encoding TetR was obtained by amplification from a template plasmid carrying the TetR sequence by means of the following oligonucleotides:

Oligo 5474 (SEQ ID No. 17) :

Page 43: remove the first, second and third paragraphs, and insert therefor:

GGCTCTAGACCCAAGCCCAGTACCCCCCCAGGTTCTTCAACGCGTGGA TCCATGTCCAGATTAGATAAAAGTAAAG

Oligo 5475 (SEQ ID No. 18):

CGTACGGAATTCGGGCCCTTACTCGAGGGACCCACTTTCACATTTAAG TTG

These oligonucleotides also provide the sequence encoding the Hinge peptide arm linking the two functional domains of the molecules.

The amplified fragment therefore contains the sequence encoding the peptide arm and the domain for binding to the tetR DNA. This fragment was cloned into the XbaI-EcoRI sites of the plasmid obtained above in order to generate a plasmid containing the sequence encoding the molecule ScFv-myc-Hinge-TetR (Figure 6).

The sequence encoding Cro was obtained by amplification on a DNA template of the lambda bacteriophage by means of the following oligonucleotides:

g14 cont'd

Oligo 5531 (SEQ ID No. 19) :

GGCTCTAGACCCAAGCCCAGTACCCCCCCAGGTTCTTCAACGCGTGGA TCCATGGAACAACGCATAACCCTGAAAG

Oligo 5532 (SEQ ID No. 20)

CGTACGGAATTCGGGCCCTTACTCGAGTGCTGTTGTTTTTTTGTTACTCGG

Page 45: remove the first paragraph and insert therefor:

g15

The cDNA encoding the oligomerizing region of the p53 protein (SEQ ID No. 3) was obtained by PCR amplification on a plasmid carrying the cDNA of the human wild-type p52 with the aid of the following oligonucleotides:

Oligo 5535 (SEQ ID No. 21) :

CAGGCCATGGCATGAAGAAACCACTGGATGGAGAA

(the underlined part represents an NcoI site)

Oligo 5536 (SEQ ID No. 22) :

CGTCGGATCCTCTAGATGCGGCCGCGTCTGAGTCAGGCCCTTC

(Underlined part : BamHI site; double underlined: XbaI site: bold: NotI site).

Page 46: remove second paragraph, and insert therefor:

4.5. The plasmids tetR or Cro-p53 320/393 (Figure 5D) or tetR or Cro-Hinge-p53 320/393 (Figure 5E) were obtained by cloning fragments amplified by PCR on a plasmid carrying the cDNA for the wild-type human p53 with the aid of the oligos 5537/5539 or 5538/5539 digested with XhoI/EcoRI into the plasmids described in 3.1, previously digested with XhoI/EcoRI.

Oligo 5537 (SEQ ID No. 23) :

CAGGCTCGAGAAGAAACCACTGGATGGAGAA

Oligo 5538 (SEQ ID No. 24) :

CAGGCTCGAGCCCAAGCCCAGTACCCCCCCAGGTTCTTCAAAG AAACCACTGGATGGAGAA

Oligo 5539 (SEQ ID No. 25) :

GGTCGAATTCGGGCCCTCAGTCTGAGTCAGGCCCTTC

Page 47: remove first paragraph and insert therefor:

Using a plasmid carrying the cDNA for the human wild-type p53 with the aid of the oligos 5661/5662 and then digested with NcoI/NotI, NcoI/XbaI, NcoI/BamHI into the plasmids described in 3.1., predigested with NcoI/NotI, NcoI/XbaI or NcoI/BamHI.

Oligo 5661 (SEQ ID No. 26) :

CAGGCCATGGAGGAGCCGCAGTCAGATCC

Oligo 5662 (SEQ ID No. 27) :